

Subj 101 → --91. The chimeric embryo according to Claim 1, wherein said chimeric embryo is an aggregation of pluripotent cells of said first animal species and said one or more second animal species, wherein said pluripotent cells are aggregated under conditions in which a viable embryo forms.--

C15 → --92. The chimeric embryo according to Claim 1, wherein said chimeric embryo is an aggregation of a mix of pluripotent cells and totipotent cells of said first animal species and said one or more second animal species, wherein said mix of pluripotent cells and totipotent cells are aggregated under conditions in which a viable embryo forms.--

REMARKS

Applicant acknowledges receipt of the Office Action mailed August 7, 2000. Applicant recognizes the extensive review and discussion the Office has devoted to this application and appreciates the extraordinary level of effort devoted by the Examiner and the Office to the application. As recognized by the Examiner, the application is significant in terms of its public policy implications, as well as its own scientific merits.

Claims 1-7, 10, 13, 16, 28-34, 38-48, 50, 53, and 55-71 are present in this application. By this Amendment, Claims 1, 3, 4, 6, 7, 10, 13, 28, 30, 31, 33, 34, 39-43, 53, 59, and 70 are amended, Claims 2, 5, 16, 29, 32, 44-48, and 56-58 are canceled without prejudice, and new Claims 72-92 are added. Applicant respectfully requests that the Examiner reconsider the pending claims, withdrawal the rejection, and allow the claims in view of the amendments and additional information submitted in this Response. New claims are added to more accurately describe the invention.

The application as originally drafted and subsequently amended claimed various permutations of a chimeric embryo. More specifically, claims were drawn to chimeric embryos, and cell lines and animals derived from chimeric embryos. The Office has rejected the claims on five separate grounds:

- First, the Examiner has rejected Claims 1-7, 13, 16, 28-34, 39-48, and 56-71 under 35 U.S.C. § 101 as being directed to non-statutory subject matter, namely as falling within an exception to statutory subject matter as embracing a human being.
- Second, the Examiner has rejected Claims 10, 13, 16, 28, 29, 32-34, 50, and 66-71 under 35 U.S.C. § 102(b) as being anticipated by certain references disclosing the introduction of human hematopoietic cells into mice *in utero* and mouse primordial germ cells and human fetal and embryo cell lines, and humans in which baboon organs were transplanted.
- Third, the Examiner has rejected Claims 1, 2, 5, 16, 28, 29, 32-34, 38-48, 56, 57, and 59-65 under 35 U.S.C. § 103(a) as being obvious, in view of a reference disclosing sheep/goat chimeras and chimeric sheep/goat pregnancies.
- Fourth, the Examiner has rejected all claims under 35 U.S.C. § 112, first paragraph, based on the assertion that the specification would not enable one of ordinary skill in the art to practice the invention, citing unpredictable outcomes in chimera formation, and the lack of fecundity of chimeric animals.
- Fifth, the Examiner has rejected all claims under 35 U.S.C. § 112, second paragraph as being vague and indefinite as to what would be considered a chimeric embryo.

The subject matter of the appended claims is made by the intervention of man. The claimed subject matter is not naturally occurring and constitutes patentable subject matter under Section 101. The vast array of references cited by the Examiner establishes the level of background knowledge of persons of ordinary skill in the art. In the context of that knowledge, the specification satisfies the requirements of Section 112. The techniques that are needed to make and use the claimed invention are well within the ordinary level of skill in the art as evidenced by the multiple references identified by the Examiner in the Office Actions. In spite of the comprehensiveness of the art, no one has practiced, taught, or suggested the use of these well known and amply documented techniques to make the claimed invention. The Examiner has recognized the richness of the level of ordinary skill, yet, has identified no reference teaching or suggesting the claimed invention as a whole.

I. The Application Claims Patentable Subject Matter (35 U.S.C. § 101)

Claims 1-7, 13, 16, 28-34, 39-48, and 56-71 are rejected under 35 U.S.C. § 101 as directed to nonstatutory subject matter. This rejection is respectfully traversed.

The Examiner maintains her earlier position that the pending claims encompass or "embrace" human beings. The Examiner cites *Diamond v. Chakrabarty*, 447 U.S. 303 (1980) and *State Street Bank & Trust Co. v. Signature Financial Group*, 149 F. 3d 1368 (Fed. Cir. 1998). The Examiner maintains that absent express intent that Congress enacted Section 101 to cover human beings as eligible for patenting, such intent cannot be inferred. The Examiner states that an embryo with human cells, but for a small percentage, or perhaps of one cell type, is considered human.

Applicant has amended the claims in an attempt to more accurately describe what Applicant considers to be the invention and to overcome the current rejection. Claims 10, 38, 50, 53, and 55

were not rejected as "embracing" a human being. The rejection has been applied against only the embryo claims.

The Examiner asserts that the PTO has rejected claims that encompass a human being under 35 U.S.C. § 101, and requires that claims drawn to animals be expressly limited to "non-human" animals. Despite the lack of an express exception for human beings in § 101, the Examiner asserts that a claim that encompasses a human being is drawn to non-statutory subject matter. Applicant maintains that the subject matter claimed in the present invention is not a human being and that no statutory authority supports the rejection on these grounds.

The rejection is improper for two reasons: (1) it is not a proper statutory requirement for patentability; and (2) the claimed subject matter is not a human being but rather, man-made chimeric cell lines, embryos and animals developing from them. Applicant respectfully submits that the Commissioner has no authority to reject the claims of the present invention--that are explicitly "made by man"--on the grounds that they "embrace a human being."

As to the first point, the only issue is whether or not the claimed invention describes *statutory* subject matter. Nowhere does the statute restrict patentability based upon embracing a human being.

The Examiner recognizes that the Court in *Chakrabarty* held that statutory subject matter shall "include anything under the sun that is made by man." (447 U.S. at 309). The claimed subject matter is not naturally occurring. It is not disputed by the Examiner that the claimed subject matter is "made by man." Applicant claims a chimeric embryo, a cell line, or chimeric animal derived from the chimeric embryo. A human being is not claimed.

The Federal Circuit recently emphasized in *State Street Bank* that neither courts nor the Patent Office are authorized to embellish the statutory requirements for patentability. The Federal

Circuit confronted the so-called "mathematical algorithm" and "business method" exceptions to patentability. As the "embraces a human being" exception grafted by the Examiner in this case, these exceptions enjoyed no statutory sanction. Unlike the "embraces a human being" exception, they enjoyed prior judicial and Patent Office application in varying degrees.

As the Federal Circuit has held so clearly in *State Street*, "any" invention "made by man" is patentable subject matter. It is for Congress--not the courts or the PTO--to set forth any limitations on patentable subject matter. Congress has not established any limitation based on subject matter that "embraces a human being." The Commissioner lacks the authority to impose one under Section 101. Whether or not the PTO believes Congress intended to bar patentability of inventions that embrace a human being is not the issue. Congress has not done so expressly and the PTO has no authority to fill that gap.

Second, the Supreme Court has held that embryos, even those consisting exclusively of human cells, are not constitutionally protected as human beings (*see, Roe v. Wade*, 410 U.S. 113 (1973)). Congress--in spite of almost thirty years of vigorous public debate--has indicated no intention of altering this holding. That holding is mandatory authority and precludes the Examiner's finding that a single cell is sufficient to make a human being.

Embryos which are not exclusively human in origin, viz. the embryos of this invention, which contain human as well as animal cells, are not human beings. They do not fall under 1077 OG 24 (4/21/87). Utility of such chimeric embryos as experimental models in biomedical and developmental biological research was documented in the original application.

Third, the present rejection is novel and unprecedented. As noted by the Examiner, mice and sheep have been engrafted with human bone marrow cells, and have been raised in laboratories as

subjects of scientific investigations (Pixley et al., (1994) *Pathobiology* **62**, 238-44; Almeida-Porada et al., (1996) *Exp. Hematol.* **24**(3), 482-7.

Pixley et al. established long term chimerism in normal mice transplanted *in utero* with human fetal hematopoietic stem cells. These human cells were injected into fetal mouse peritoneal cavities on days eleven through thirteen of gestation. These animals may develop and contain human cells in various organs. This engraftment of human cells into mouse fetuses does not now qualify the mouse as a human being, nor does it create a human being. The Office has never held that it does prior to the present invention and has regularly granted patents on such inventions.

Almeida-Porada et al. describes the transplantation *in utero* of preimmune fetal sheep with human hematopoietic stem cells which result in a long term chimerism. These experiments reported the long term persistence of human cells in the human/sheep xenograft model. As with the above, the sheep, although containing human cells, are not considered human beings.

While Applicant disputes the Examiner's claim that *Pixley et al.* represents prior art with respect to the present invention (see below), it is clear that these organisms represent "animals containing human cells." They are not constitutionally protected as human beings. Because specific utility of non-human animals containing human cells, constructed by the methods of this invention, was documented in the original application, such organisms do not fall under 1077 OG 24 (4/21/87).

Applicant respectfully submits that a proportion of human cells in an organism does not make that organism a human being. In addition, the original application, and subsequent amendments, do not include any claims to a human being, but only contains claims to a chimeric embryo, a cell line, or a chimeric animal isolated or originating from the chimeric embryo. The fact that a chimeric embryo, a cell line, or a chimeric animal has a human cellular component cannot exclude it from

patentability, any more than the many patents that share that feature and have been awarded by the PTO. Subject matter consisting of, or derived from, human cells in non-human animal systems has been, and continues to be, granted patents in the area of biotechnology.

Applicant maintains its position that it is not making a human being, or anything that "embraces a human being." Applicant's invention is a chimeric embryo, a cell line, or a chimeric animal that is developed in the laboratory and made by man. The "right to exclude others from making the invention" is the right to exclude others from making these embryos as described in the application. While each application is evaluated on its own facts, the PTO must provide a consistent interpretation as to what is patentable subject matter and what is not. Inventors routinely consult issued patents for guidance in determining the patentability of their own inventions.

The only issue is whether or not the claimed invention describes *statutory* subject matter. Nowhere does the statute restrict patentability based upon embracing a human being. Reconsideration and withdrawal of the rejection is respectfully requested.

II. The Claims Define Patentable Subject Matter

A. The Claims are Patentable over Pixley et al.

Claims 28, 29, and 32-34 are rejected under 35 U.S.C. § 102(b) as being anticipated by Pixley et al. (1994) *Pathobiol.* **62**, 238-244. This rejection is respectfully traversed.

The Examiner contends that the organisms disclosed by Pixley, et al., i.e. xenografts, xenotransplants, or even allografts or allotransplants, fall squarely into the definition of a "chimera". In addition, the Examiner contends that the disclosure of Pixley, et al. continues to anticipate the pending claims as the rejected claims include the scenario where the second animal species is mouse.

Applicant has amended the claims to more accurately define the invention. Claim 28 is amended to delete the terms "domestic pig, mouse, rat, and rabbit". Claim 28 is also amended to include embryonic cell types and that the embryonic cells must cooperate to form the chimeric embryo. Support for the claim amendments may be found in the specification as follows: p.1, ln.18; p.2, lns.9-15, 20; p.4, lns.4-7; p.5, lns.4-5; p.16, lns.1-3; p.17, lns.17-19; p.18, ln.21; p.19, ln.12; and p.1, lns.19-20; p.6, lns.17-18, 22; p.16, lns.3-4; p.19, lns.2-5, 12-15, 19; p.20, lns.4-5, 19-20, respectively.

The Examiner states that the invention was anticipated by the description by Pixley et al. (1994) of the introduction of human hematopoietic cells into mice *in utero*. Pixley, J. S., Zanjani, E. D., Shaft, D. M., Porada, C., and Mackintosh, F. R. (1998). Prolonged Hematopoietic Chimerism in Normal Mice Transplanted *in utero* with Human Hematopoietic Stem Cells. *Pathobiology* **66**, 230-9) conducted late embryo grafting experiments to produce hematopoietic organisms, i.e., mixtures of blood forming cells in an organism (mouse) that is unambiguously of one species. The claims are amended to limit the Applicant's invention to the use of human and non-human primate cells.

The present invention claims an embryo, cell line, or embryo developing into an organism that is a true embryo-based chimera. Fehilly, et al., (1984) and Meinecke-Tillmann and Meinecke (1984) described true embryo chimeras that exhibit composite morphology and multi-tissue chimerism. This definition is generally accepted in the field by one of ordinary skill in the art. True embryo chimeras do not result from xenografts or transplants of adult or differentiated cells. See Declaration of Dr. Martha Herbert, attached as Exhibit A. The work of the Pixley et al. group represents xenograft models. None anticipates the claimed invention.

Applicant has amended the claims to he use of embryonic cells in the formation of the chimeric embryo, cell line, and chimeric animals. The chimeric embryo of the present invention is not intended to include fetal stage xenografts or any entity constructed with cells of developmental stages later than the inner cell mass.

The PTO is defining chimera as "an organism made up of two or more tissues of different genetic composition". Applicant respectfully rejects this definition. The PTO's definition would have any graft or transplant patient considered a chimera. This is clearly not the case. In the widely-used textbook *Developmental Biology* (Sinauer, 1997), well known cell biologist Scott Gilbert defines "chimeric mice" as "the result of two or more early cleavage (usually 4- or 8-cell) embryos that have been artificially aggregated to form a composite embryo." These embryo aggregation chimeras, which as Gilbert points out, can also be produced with mixtures of embryo cells and embryo stem (ES) cells, clearly correspond to our invention, and not to the animals generated by Pixley et al.

The present invention and amended claims describe chimeric embryos containing human and non-human primate cells, where aggregation of embryonic cells (i.e., blastomere cells, blastocyst cells, undifferentiated immortal cells, pluripotent cells, totipotent cells, and embryonic stem cells) of two or more species is performed. This is entirely different, and leads to different developmental outcomes, than the engraftment of **multipotent** stem cells during fetal stages as described by Pixley et al.

Applicant respectfully submits that Pixley et al. fails to disclose the subject matter of the claimed invention. Reconsideration and withdrawal of the rejection is respectfully requested.

B. The Claims are Patentable over the ATCC Catalog of Cell Lines and Hybridomas.

Claims 10, 50, and 68 are rejected under 35 U.S.C. § 102(b) as being anticipated by the Catalog of Cell Lines and Hybridomas, 7th ed., American Type Culture Collection (ATCC), Rockville, MD. 20852-1776, 1992, entry HTB 157, HTB 158, and HTB 160, page 271, and cell line CRL2378. The rejection is respectfully traversed. The Examiner states that the existence of human cell lines in the American Type Culture Collection anticipates the present invention.

Claims 10 and 68 are rejected under 35 U.S.C. 102(b) as anticipated by ATCC entries HTB 157, 158, and 160, p. 271. The Examiner maintains that any immunological differences exhibited by cells derived from chimeras would depend upon the cell type.

Cells derived from chimeras are known to differ in immunological properties from equivalent cells in non-chimeric animals. One of ordinary skill in the art would expect that this would also likely pertain to cell lines derived from chimeras. Applicant does not completely understand the PTO's continued rejection under 35 U.S.C. § 102(b) as being anticipated by the American Type Culture Collection Catalogue of Cell Lines. Applicant has amended the claims to more accurately describe the present invention, including embryonic cell types and tolerance of the claimed cell line to cells from the first and the one or more second animal species. Support for the amendments may be found in the specification as follows: p.1, ln.18; p.2, lns.9-15, 20; p.4, lns.4-7; p.5, lns.4-5; p.16, lns.1-3; p.17, lns.17-19; p.18, ln.21; p.19, ln.12; and p.10, lns. 21-22; p.11, lns. 1, 7-20; p.14 lns. 1-2; p.20, lns. 3-22, respectively.

Embryo aggregation chimeras produced according to an embodiment of the present invention would be expected to be immunologically unprecedented in that they would likely be tolerant to

grafts from both human and the nonhuman species used, but not from other species. The claims have been amended to reflect this property. For example, Gustafson, et al., (1993) tested four sheep-goat chimeras with a goat or sheep sibling having an identical genotype to one of the two component species of cells for tolerance through mixed lymphocyte response (MLR) and skin grafts. None of the four chimeras showed a response to its sibling in MLR and three of the four accepted sibling skin grafts. This demonstrates that the chimerism exhibited by these animals was sufficient to render the chimera tolerant to antigens expressed by the sibling (Gustafson, R. A., Anderson, G. B., BonDurant, R. H., and Mahi-Brown, C. (1993). Tolerance of Sheep-Goat Chimeras to their Component Cells. *J Reprod Immunol* 23, 155-68).

Applicant's original specification on page 10 discusses rejection of xenografts in non-chimeric animals due to endothelial cell activation, with relevant references. However, it is known that chimeric animals tolerate xenografts from their originating species (Gustafson, 1993). This is reasonably related to the induction of protective genes in the chimeric animal's endothelium which limit the activation process (Bach FH, Hancock WW, Ferran C (1997) Protective genes expressed in endothelial cells: a regulatory response to injury. *Immunol Today* 18, 483-6). This in turn provides a motivation to produce endothelial cell lines, such as have been used in studies of xenograft rejection (Malassagne B, Taboit F, Conti F, Batteux F, Atia N, Chereau C, Conjeaud H, Theron MC, Attal J, Braet F, Houdebine LM, Calmus Y, Houssin D, Weill B (1998) A newly established porcine aortic endothelial cell line: characterization and application to the study of human-to-swine graft rejection *Exp Cell Res* 238, 90-100) from the chimeric animals of the invention, to explore the endothelial determinants of xenograft rejection.

In a commonly used model of interspecies grafting, the athymic, or nude, mouse, grafts from

other species are tolerated, but only because the mouse's cellular immunity is entirely compromised. The chimeric model of the present invention is different—there is tolerance to the originating species because the relevant antigens were present during development. This model would therefore be highly useful in understanding the genesis of the tissue immune response in humans, and accelerate the development of anti-graft rejection human therapeutics.

Claims 10, 50, and 68 are rejected under 35 U.S.C. 102(b) as being anticipated by ATCC, entry CRL-2378, designated MA-104. The Examiner contends that the breadth of Applicant's claims would encompass a cell line isolated from embryonic kidney tissue of a Rhesus monkey, as cells isolated and used to generate a cell line may not include both species.

Applicant has amended the claims to more accurately define the present invention, as discussed above.

Applicant respectfully submits that the Catalog of Cell Lines and Hybridomas fails to disclose the subject matter of the claimed invention. Reconsideration and withdrawal of this rejection is respectfully requested.

C. The Claims are Patentable over Starzl et al.

Claims 13, 66, 67, and 69-71 are rejected under 35 U.S.C. 102(b) as being anticipated by Starzl et al. This rejection is respectfully traversed. Starzl et al. disclose humans in which baboon kidneys or livers were transplanted and resulted in chimerism of the patient. The Examiner contends that the breadth of the claims encompass the organisms disclosed by Starzl et al., and, as such, the claims are rejected.

Applicant has amended the claims to more accurately describe the present invention, including embryonic cell types and tolerance of the claimed chimeric animal to cells from the first

and the one or more second animal species. Support for the amendments may be found in the specification as follows: p.1, ln.18; p.2, lns.9-15, 20; p.4, lns.4-7; p.5, lns.4-5; p.16, lns.1-3; p.17, lns.17-19; p.18, ln.21; p.19, ln.12; and p.10, lns. 21-22; p.11, lns. 1, 7-20; p.14 lns. 1-2; p.20, lns. 3-22, respectively.

The human-animal chimeras disclosed by Starzl et al. are different from those of the invention since they are the result of adult tissue cells of one species colonizing adult tissues of another species, rather than the result of developmental cooperation of early embryonic cel types of different species. See Declaration of Dr. Martha Herbert, attached as Exhibit A. Thus, for example, the "geeps" made from early embryo cells of sheep and goats (Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (1984). Interspecific chimaerism between sheep and goat. *Nature* **307**, 634-6; Meinecke-Tillmann, S., and Meinecke, B. (1984). Experimental chimaeras--removal of reproductive barrier between sheep and goat. *Nature* **307**, 637-8) had phenotypic and morphological properties of both species, as well as histologically indentifiable cells of numerous different types from both species in single individuals, evidencing synergistic development. All other embryo chimeras produced by the embryo chimera methods diecribed in the application similarly exhibit multitissue and phenotypic chimerism (Gardner, R. L. (1968). Mouse chimeras obtained by the injection of cells into the blastocyst. *Nature* **220**, 596-7; Rossant, J., and Chapman, V. M. (1983). Somatic and germline mosaicism in interspecific chimaeras between *Mus musculus* and *Mus caroli*. *J Embryol Exp Morphol* **73**, 193-205; Picard, L., Chartrain, I., King, W. A., and Betteridge, K. J. (1990). Production of chimaeric bovine embryos and calves by aggregation of inner cell masses with morulae. *Mol Reprod Dev* **27**, 295-304; Piedrahita, J. A., Gillespie, L., and Maeda, N. (1992).

Production of chimeric hamsters by aggregation of eight-cell embryos. *Biol Reprod* **47**, 347-54; Williams, T. J., Munro, R. K., and Shelton, J. N. (1990). Production of interspecies chimeric calves by aggregation of *Bos indicus* and *Bos taurus* demi-embryos. *Reprod Fertil Dev* **2**, 385-94). The human patients described by Starzl et al., who had received baboon hearts or livers, or chimpanzee kidneys *via* transplantation, were not reported to exhibit any morphological or phenotypic similarities to baboons or chimpanzees, indicating lack of synergistic development, nor did they exhibit any tissue level chimerism other than that due to leukocyte transfusion from the donor species.

The animals of Starzl et al. are not considered to originate from the chimeric embryos as disclosed and claimed in the present invention. Applicant has amended the subject claims to more accurately describe the present invention.

Applicant respectfully submits that Starzl et al. fails to disclose the subject matter of the claimed invention. Reconsideration and withdrawal of this rejection is respectfully requested.

D. The Claims are Patentable over humans or non-human primates as found in nature

Claim 16 is rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over human or non-human primates as found in nature. The rejection is respectfully traversed.

Claim 16 is directed to a descendant of the chimeric animal of Claim 13. The Examiner contends that the descendant of a chimeric animal is not necessarily any different from one of the source species.

The chimeras themselves would be expected to have endothelial cells with different gene expression profiles from either of the originating species, even though each endothelial cell is

genetically one or the other species. It is reasonable to expect that this property of the endothelium would be propagated through the germline, leading to decendent organisms of one or the other species that have altered endothelial properties. Nonetheless, Applicant cancels Claim 16 without prejudice.

E. The Claims are Patentable over Gustafson et al.

Claims 1, 2, 5, 28, 29, 32-34, 38-48, 56-57, and 59-65 are rejected under 35 U.S.C. § 103 as being unpatentable over Gustafson et al. (1993) *J. Reprod. Fert.* **99**, 267-273. This rejection is respectfully traversed.

The Examiner contends that because Gustafson et al. discloses sheep-goat chimeras, the art itself in combination with Gustafson et al. would motivate one with ordinary skill in the art to make aggregates including human cells. The Examiner contends that motivation does not have to be found specifically in a prior art reference, but can be taken from the general state of the art itself.

Applicant has amended the claims to more accurately define the present invention. Claims 1 and 28 are amended to include embryonic cell types and that the embryonic cells must cooperate to form the chimeric embryo. Support for the claim amendments may be found in the specification as follows: p.1, ln.18; p.2, lns.9-15, 20; p.4, lns.4-7; p.5, lns.4-5; p.16, lns.1-3; p.17, lns.17-19; p.18, ln.21; p.19, ln.12; and p.1, lns.19-20; p.6, lns.17-18, 22; p.16, lns.3-4; p.19, lns.2-5, 12-15, 19; p.20, lns.4-5, 19-20, respectively.

The Examiner asserts that Gustafson et al. provides the motivation to make chimeric embryos containing human cells. The abstract of that paper is as follows:

Six hybrid pregnancies were established: three in sheep-goat chimaeras, one in a sheep-(sheep-goat)hybrid chimaera and two in does. Pregnancies were monitored weekly by ultrasonography and

peripheral concentrations of pregnancy specific protein B (PSPB) were measured. Placental development as detected by ultrasonography appeared to be slower in hybrid-in-goat pregnancies than in hybrid-in-chimaera pregnancies, although this difference was not reflected in PSPB concentrations. Time of fetal death could not be predicted from PSPB concentrations. Chimaeras appeared to carry hybrid pregnancies longer than ewes and does usually carry hybrid pregnancies, but none was carried to term.

Although this paper utilized chimeras to study pregnancy retention and placental development, i.e., some of the things that might be studied if chimeric animals containing human and nonhuman cells were available, as per an embodiment of the present invention, the issues and discussion are very far from anything involving human biology. See Declaration of Dr. Martha Herbert, attached as Exhibit A. This paper has been cited twice in the literature indexed in the Scientific Citations Index ("SCI") since the time it was published. These citations are: Slavik, T., Kopecny, V. and Fulka, J. (1997) Developmental Failure of Hybrid Embryos Originated after Fertilization of Bovine Oocytes with Ram Spermatozoa. *Molec. Reprod. Develop.* **48**, 344-349, and Willard, S. T., Sasser, R. G., Jaques, J. T., White, D. R., Neuendorff, D. A., and Randel, R. D. (1998). Early-pregnancy Detection and the Hormonal Characterization of Embryonic-Fetal Mortality in Fallow Deer (*Dama-Dama*). *Theriogenology* **49**, 861-869. Neither reflects the motivation attributed to Gustafson, et al., by the Examiner.

The present invention describes embryos produced from embryo or ES cells from two different primate species, one of which is a human. This is entirely different from the disclosure of Gustafson et al., 1993, whose work, as evident from the first sentence of their paper ("The domestic sheep *Ovis aries* and the domestic goat *Capra hircus* do not normally interbreed"). The paper of Gustafson et al., is solidly within the scientific field of animal husbandry, where the issue of

hybridization is important, leading, for example, to mules. Investigators in this field might be motivated to apply Gustafson's teachings on sheep and goats to cattle, swine, horses, etc. Because primates are not domesticated farm animals, readers of papers like Gustafson et al. would not be motivated to consider this group of animals as potential subjects of such investigations. Thus shifting consideration to primates represents one major displacement from the area of teaching of Gustafson. Secondly, one of the species in the invention is designated as human. But within the field of primate biology there is a teaching away, for cultural and social reasons, from consideration of overcoming reproductive barriers between nonhuman species and humans. In the 41 articles turned up by Medline searches for "human nonhuman breeding" and "human nonhuman hybrid" there is not a single one that contemplates the kind of interspecies hybridization that Gustafson et al., 1993 was exploring with the chimera technique. Correspondingly, there is nothing in the scientific literature that supports the existence of a barrier to hybridization between human and chimpanzee, or human and gorilla. Thus the main motivation for the work of Gustafson et al. is not present in the case of human and nonhuman primates. The instant invention is therefore doubly displaced from the area of art represented by Gustafson et al.—it exclusively concerns primates, a class of animals that readers of this paper would not be working on. Moreover it considers human-nonhuman mixtures, which, if one were to follow Gustafson et al., would be primarily achieved by hybridization, which is taught away from.

Persons who have relied upon Gustafson have not been so motivated. The Examiner has provided no reference that teaches or suggests chimeric embryos containing human cells. Examiner maintains the rejection based upon the ability of the embryo to form a cooperative entity. Applicant submits that there was actually a teaching away of the formation of chimeric embryos containing

human cells. The only references relying upon Gustafson did so in the context of embryo mortality, not formation. As the references cited by the Examiner establish, the area of developmental biology is unpredictable, making the utility of the present invention so powerful. It is for this reason, among others, that the claimed subject matter is not obvious.

Applicant submits that it would not be obvious under Gustafson et al. for one of ordinary skill in the art at the time of the invention to make a cell aggregate comprising human cells. The Examiner contends that an invention is capable of being unpredictable, yet obvious. The Examiner also contends that the unpredictability is based upon the ability of the embryo to form a cooperative entity. Applicant has amended the claims to indicate that the embryonic cells used to form the chimeric embryo must cooperate, not merely aggregate, to form a viable embryo.

Applicant maintains that Gustafson et al. does not provide the motivation or teaching to make chimeric embryos with human cells. Reconsideration and withdrawal of this rejection is respectfully requested.

III. The Claims Satisfy 35 U.S.C. § 112, First Paragraph

Claims 1-7, 10, 13, 16, 28-34, 38-48, 50, 53, and 55-71 are rejected under 35 USC § 112, First Paragraph for lack of adequate enabling disclosure. This rejection is respectfully traversed.

The Examiner has taken the position that the specification fails to provide an enabling disclosure for how to make and use the claimed invention. Applicant respectfully submits that the claim amendments and remarks presented in this Response obviate the grounds for the rejection. Reconsideration and withdrawal of the rejection are respectfully requested.

Claims 39-48 and 55 stand rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The Examiner maintains that if a claim specifically recites a limitation on viability, the limitation must be disclosed in the specification. The fact that the specification states that the embryo can be propagated for varying periods, does not enable claims that recite a specific limitation, i.e., a specific time.

Applicant, on Page 5 of the original specification, states "Once chimeric embryos are produced they can be propagated for varying periods of time in culture, where they may undergo a series of developmental steps . . . For some uses, the embryos can be brought to term, forming the chimeric animals of the invention." The claims are amended to reflect this disclosure. Applicant respectfully submits that it is well known in the art that chimeric organisms may or may not cease to be viable at any given time.

In an effort to more clearly define the present invention, Applicant has either amended or canceled the subject claims. Applicant has amended Claims 39-43 to claim the chimeric embryo at a particular stage of development, and Claims 44-48 are canceled without prejudice.

Claims 56 and 57 are rejected under 35 U.S.C. 112, first paragraph. The Examiner contends that the specification does not disclose that the chimeric embryo would exhibit composite morphology or multi-tissue chimerism. The Examiner states that these limitations constitute new matter and that the specification does not provide adequate written description of the claimed invention.

Applicant respectfully submits that the description of a chimeric embryo as found in Gilbert's *Developmental Biology* (Sinauer, 1997): "the result of two or more early cleavage (usually 4- or 8-cell) embryos that have been artificially aggregated to form a composite embryo" (p. 187) and as being made from early stage embryo cells (blastomeres) and embryo stem (ES) cells (189), and as defined by Papaioannou and Gardner, as being made from early cleavage embryos and inner cell mass cells (Papaioannou, V., and Gardner, R. L. (1979). Investigation of the lethal yellow Ay/Ay embryo using mouse chimaeras. *J Embryol Exp Morphol* **52**, 153-63), obviates the ground for this rejection. In every case in the scientific literature in which chimeric embryos were made according to these specifications multi-tissue chimerism resulted. In every case in the literature in which dual species chimeric embryos were made according to these specifications, composite morphology also resulted. It would therefore be evident to one of ordinary skill in the art that producing dual or multi-species chimeric embryos according to this commonly accepted definition would imply multi-tissue chimerism and composite morphology.

Applicant respectfully argues that those traits are inherent in the organisms of the present invention as described by Applicant in the specification. Nonetheless, Applicant has canceled Claims 56 and 57 without prejudice.

Claims 1-7, 10, 13, 16, 28-34, 38-48, 50, 53, and 55-71 are rejected under 35 U.S.C. 112, first paragraph. The Examiner contends that the specification does not disclose the essential features of the claimed chimeras and that the specification describes the chimeras as having an unspecified degree of chimerism. The Examiner continues that the specification does not disclose what contribution each species would make to the chimera and that the written description is insufficient to inform a skilled artisan that Applicant was in possession of the claimed invention as a whole at

the time the application was filed. The Examiner contends that the specification contains no specific description of the ultimate structure, physical and anatomical, of the chimeras, and that the contribution that each species makes to the chimera is not set forth. The Examiner maintains that the skilled artisan cannot envision the detailed structure of the encompassed chimeras, cell lines, or animals, and therefore conception is not achieved.

It is clear from the literature that species that are much more dissimilar than human and chimp or human and gorilla cooperate to produce a coherent organism (Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (1984). Interspecific chimaerism between sheep and goat. *Nature* **307**, 634-6; Meinecke-Tillmann, S., and Meinecke, B. (1984). Experimental chimaeras--removal of reproductive barrier between sheep and goat. *Nature* **307**, 637-8). Specifically, sheep and goats are biologically much more distant from one another (Randi, E., Fusco, G., Lorenzini, R., Toso, S., and Tosi, G. 1991. Allozyme divergence and phylogenetic relationships among Capra, Ovis and Rupicapra (Artyodactyla, Bovidae). *Heredity* **67**, 281-6) than human and chimpanzee (Takahata, N., and Satta, Y. 1997. Evolution of the primate lineage leading to modern humans: phylogenetic and demographic inferences from DNA sequences. *Proc Natl Acad Sci U S A* **94**, 4811-5). The chimpanzee has been known from the early 1980 to be an excellent model system for in vitro fertilization and early embryogenesis in humans because of the extensive similarities in early embryogenesis in these species (Gould, K. G. (1983). Ovum recovery and in vitro fertilization in the chimpanzee. *Fertil Steril* **40**, 378-83). Moreover, humans and chimpanzees are reproductively similar, having similar menstrual cycles with bleeding (Strassmann, B. I. (1996). The evolution of endometrial cycles and menstruation. *Q Rev Biol* **71**, 181-220), placentas with similar tissue and molecular structure (Soma, H. (1983). Notes on the morphology of the chimpanzee and orang-utan

placenta. *Placenta* **4**, 279-90), and similar levels of chorionic gonadotropin (Hobson, B. M. (1975). Chorionic gonadotropin in the placenta of a chimpanzee (*Pan troglodytes*). *Folia Primatol (Basel)* **23**, 135-9). One skilled in the art would fully expect that embryo chimeras constructed from human blastomeres, inner cell mass cells, or ES cells and chimpanzee or gorilla blastomeres, inner cell mass cells, or ES cells would have at least as the degree of multitissue chimerism and composite morphology as that reported for sheep and goat.

Claims 1-7, 10, 13, 16, 28-34, 38-48, 50, 53, and 55 stand and newly presented Claims 56-71 are, rejected under 35 U.S.C. 112, first paragraph for failing to teach how to make and use the invention. The Examiner does not agree with Applicant's statement that the technology for producing chimeric mammalian embryos is "robust". The Examiner contends that only a very few species have been used in making chimeric embryos, and that none of the prior art methods enable one to culture primate embryos. The Examiner maintains that it is unpredictable as to whether the culture methods used in mouse, rat, sheep, or goats could be extrapolated to primate embryos. Though one of skill in the art could easily mix together embryonic cells of two species, the formation of a cooperative entity and its viability for any length of time is completely unpredictable. Therefore, the Examiner maintains that the specification does not enable how to make the claimed embryos, cell lines, or animals.

The techniques for manipulating mammalian embryos are clearly robust and enabling for the production of chimeras. See Declaration of Dr. Martha Herbert, attached as Exhibit A. For example Hammer states in a 1998 retrospective (Hammer, R. E. (1998). Egg culture: the foundation. *Int J Dev Biol* **42**, 833-9):

In 1963, Ralph [Brinster] reported a method for culturing eggs in microdrops of medium under oil (Brinster, 1963), which has become universally used. Two years later, he identified pyruvate as the central and essential energy source for early stages of mouse eggs (Brinster, 1965b). These two developments revolutionized in vitro studies of mammalian eggs and issued in an era of intense research activity concerning egg culture and egg manipulation. Effective formulations of culture media could now be developed to allow routine in vitro maintenance of eggs, and important parameters for these recipes were soon determined... **Thus, a foundation of understanding about the biology of early mammalian eggs was established between 1960 and 1970, and subsequent studies have broadened this understanding.** However, the greatest impact of a simple, reliable egg culture method has been to provide the ability to perform complicated manipulative procedures on preimplantation stages of mammalian embryos. In no area has this been more important than in development of transgenic animals. All methods for generating germ line genetic modifications rely on the ability to maintain and manipulate eggs and early developmental stages in vitro without loss of developmental competence. The importance of efficient egg culture to manipulation and transgenesis is fundamental and **enabling.** [Emphasis added].

Another recent article (Leoni, G., Ledda, S., Bogliolo, L., and Naitana, S. (2000). Novel approach to cell sampling from preimplantation ovine embryos and its potential use in embryonic genome analysis. *J Reprod Fertil* **119**, 309-14) also speaks to the robustness of mammalian embryo manipulation techniques. It states:

The major obstacle in the extensive analysis of the embryonic genome is the small number of cells typically obtained after the embryo biopsy. The object of the present study was to develop a simple approach that would allow the collection of a sufficient number of cells from a single embryo for use in further analyses... [N]o significant differences were found in the viability rates in vitro among blastocysts vitrified immediately after biopsy (77.8%), blastocysts biopsied and vitrified after 24 h culture (76.9%) and blastocysts vitrified without manipulation (88.5%). In experiments in vivo, the lambing rate of biopsied and vitrified blastocysts was significantly ($P < 0.05$) lower (40.0%) compared with vitrified control embryos (68.7%). **This new approach to the biopsy of preimplantation**

embryos is a useful good model in the assisted reproductive technologies of domestic, wild and human species. [Emphasis added].

An earlier report (Anderson, G. B. (1985). Manipulation of the mammalian embryo. *J Anim Sci* **61**, 1-13). shows that by the early 1980s the robustness of mammalian embryo manipulation techniques and their transferrability across species lines was already part of the practice of the field:

Technological advances in manipulation of mammalian embryos outside the maternal environment have resulted in opportunities for study of preimplantation embryo development, identification of developmental phenomena that are unique to mammals, and further improvement of technology. Mammalian embryos may be cultured in vitro at 37 C for up to several days or they may be stored at -196 C indefinitely. The mammalian embryo possesses the unique capacity to regulate its development and differentiate into a normal individual after being stimulated to incorporate foreign cells or after a portion of its cells are removed. **This regulatory ability has proven useful in research dealing with the production of chimeras...Some of these manipulations have been carried out primarily in laboratory mice, but as animal scientists identify beneficial uses in farm animals, these procedures are being extended to embryos of the large domestic species. [Emphasis added].**

The Examiner contends that only a very few species have been used in making chimeric embryos. Applicant respectfully submits that this is not the case: Picard, L., Chartrain, I., King, W. A., and Betteridge, K. J. (1990). Production of chimaeric bovine embryos and calves by aggregation of inner cell masses with morulae. *Mol Reprod Dev* **27**, 295-304; Onishi, A., Takeda, K., Komatsu, M., Akita, T., and Kojima, T. (1994). Production of chimeric pigs and the analysis of chimerism using mitochondrial deoxyribonucleic acid as a cell marker. *Biol Reprod* **51**, 1069-75; Schoonjans, L., Albright, G. M., Li, J. L., Collen, D., and Moreadith, R. W. (1996). Pluripotential rabbit embryonic stem (ES) cells are capable of forming overt coat color chimeras following injection into

blastocysts. *Mol Reprod Dev* **45**, 439-43; Sumantri, C., Boediono, A., Ooe, M., Saha, S., and Suzuki, T. (1997). Fertility of sperm from a tetraparental chimeric bull. *Anim Reprod Sci* **46**, 35-45.

There is also evidence for intrauterine chimerism in the human, i.e., formation of a single individual from aggregation of blastomeres of fraternal twins: De la Chapelle, A., Schroder, J., Rantanen, P., Thomasson, B., Niemi, M., Tiilikainen, A., Sanger, R., and Robson, E. B. (1974). Early fusion of two human embryos? *Ann Hum Genet* **38**, 63-75; Mayr, W. R., Pausch, V., and Schnedl, W. (1979). Human chimaera detectable only by investigation of her progeny. *Nature* **277**, 210-1.

The Examiner also states that none of the prior art enables one of ordinary skill in the art to culture primate embryos. Applicant respectfully submits that this is not the case: Pope, C. E., Pope, V. Z., and Beck, L. R. (1982). Development of baboon preimplantation embryos to post-implantation stages in vitro. *Biol Reprod* **27**, 915-23; Gould, K. G. (1983). Ovum recovery and in vitro fertilization in the chimpanzee. *Fertil Steril* **40**, 378-83; Pope, V. Z., Pope, C. E., and Beck, L. R. (1984). SP-I secretion by baboon embryos in vitro. *Placenta* **5**, 403-12; Fourie, F. R., Snyman, E., and van der Merwe, J. V. (1987). Supplementation of Ham's F10 culture medium with three different sera in the culturing of baboon oocytes. *Comp Biochem Physiol A* **87**, 1103-6 and Pope, C. E., Dresser, B. L., Chin, N. W., Liu, J. H., Loskutoff, N. M., Behnke, E. J., Brown, C., McRae, M. A., Sinoway, C. E., Campbell, M. K., Cameron, K. N., Owens, O. M., Johnson, C. A., Evans, R. R., and Cedars, M. I. (1997). Birth of a western lowland gorilla (*Gorilla gorilla gorilla*) following in vitro fertilization and embryo transfer. *Am J Primatol* **41**, 247-60 all report the culture of primate embryos.

Applicant's specification describes three specific technologies for making interspecific embryo chimeras, with citations to the published literature. The Examiner has cited dozens of

references, establishing that the techniques are not only well known in the published literature, but readily apprehended and used by researchers in the art for a wide variety of investigations.

All early mammalian embryos, including human embryos, undergo the same initial developmental steps. All go through a *two cell*, *four cell*, and *eight cell* stage, and all are initially surrounded by an extracellular layer known the *zona pellucida*. All form a hollow *blastula*, containing an *inner cell mass*. The inner cell mass further develops into two or more layers of cells known as the germinal layers. The germinal layers, the ectoderm, the mesoderm, and the endoderm, give rise to the various cell types that make up the adult animal. (An Introduction to Embryology, Fourth Edition (1975), Balinsky, B.I., W.B. Saunders Company, Philadelphia PA; Molecular Biology of the Cell, Second Edition (1989), Alberts, B. et al., Garland Publishing, Inc., New York, NY). Applicant respectfully submits that methods to create chimeric embryos were adequately disclosed and enabled the present invention. See Declaration of Dr. Martha Herbert, attached as Exhibit A.

The Examiner maintains that intraspecies chimeras of mouse/rat and sheep/goat cannot be extrapolated to human/non-human primate chimeras. The Examiner states that although human/chimp or human/gorilla may share 90 percent DNA homology; however, the DNA homology does not account for anatomical differences, differences in gestation, or more importantly how to get a host mother to carry such a chimera.

Applicant respectfully refers the Examiner to the discussion found on pages 26-27 of this Response.

Applicant respectfully contends that the existing art was sufficient at the time of filing to permit one of ordinary skill in the art to construct the chimeric embryos, cell lines, and animals of

the present invention. Reconsideration and withdrawal of this rejection is respectfully requested.

IV. The Claims Satisfy 35 U.S.C. § 112, Second Paragraph

Claims 1-7, 10, 13, 16, 28-34, 38-48, 50, 53, and 55-71 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. This rejection is respectfully traversed.

Applicant respectfully submits that the above-outlined amended claims and the remarks presented in this Response obviate the grounds for the rejection. Reconsideration and withdrawal of the rejection are respectfully requested.

The Examiner contends that the phrase "chimeric embryo" renders the claims indefinite because it does not clear when a cell aggregation becomes an embryo, and that the specification does not set forth a definition of when a cell aggregation actually is considered an embryo. The Examiner interprets the term to mean simply a mixture of cells from two individuals.

By "viable embryo," Applicant is referring to a chimeric embryo which is alive (in the sense of respiration and not necessarily progressing full term) and capable of developing to the next or successive stage of development, as the term "viable embryo" is used in the developmental and reproductive biology literature. The subject claims are amended to more accurately describe that the embryonic cells must cooperate in order to form a viable embryo. Support for the claim amendments may be found in the specification as follows: p.1, ln.18; p.2, lns.9-15, 20; p.4, lns.4-7; p.5, lns.4-5; p.16, lns.1-3; p.17, lns.17-19; p.18, ln.21; p.19, ln.12; and p.1, lns.19-20; p.6, lns.17-18, 22; p.16, lns.3-4; p.19, lns.2-5, 12-15, 19; p.20, lns.4-5, 19-20.

The Examiner states that Claim 13 is indefinite due to the recitation of the phrase "derived from" because the phraseology is unclear as to what would be required for a chimeric animal to be "derived" from a chimeric embryo. "Derived from a chimeric embryo" has an unambiguous

meaning in the scientific literature, which uses "derive" to refer to "originate from, generate from, or produce from". Even though Applicant believes the original claim language adequately describes the present invention, Applicant has amended the subject claims by replacing the term "derived" with the term "originating" to address the Examiner's concerns.

IV. Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully submits that the claims define statutory subject matter that is patentable over the art of record and the application is in condition for allowance. Should the Examiner believe anything further is desirable to place the application in better condition for allowance, the Examiner is invited to contact Applicant's undersigned attorney at the telephone number listed below.

Respectfully Submitted,

Date: February 7, 2001



PATRICK J. COYNE, Reg. No. 31,821
JOHN N. COULBY, Reg. No. 43,565
COLLIER, SHANNON, RILL & SCOTT, PLLC
3050 K Street, N.W., Suite 400
Washington, D.C. 20007
(202) 342-8400